Variations in the radiation sensitivity of foodborne pathogens associated with complex ready-to-eat food products ☆

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Received 22 February 2005; accepted 19 December 2005

Abstract

Foodborne illness outbreaks and product recalls are occasionally associated with ready-to-eat (RTE) sandwiches and other "heat and eat" multi-component RTE products. Ionizing radiation can inactivate foodborne pathogens on meat and poultry, fruits and vegetables, seafood, and RTE meat products. However, less data are available on the ability of low-dose ionizing radiation, doses under 5 kGy typically used for pasteurization purposes, to inactivate pathogenic bacteria on complex multi-component food products. In this study, the efficacy of ionizing radiation to inactivate *Salmonella* spp., *Listeria monocytogenes*, *Staphylococcus aureus*, *Escherichia coli* O157:H7, and *Yersinia enterocolitica* on RTE foods including a "frankfurter on a roll", a "beef cheeseburger on a bun" and a "vegetarian cheeseburger on a bun" was investigated. The average D-10 values, the radiation dose needed to inactivate 1 $\log_{10}$ of pathogen, by bacterium species, were 0.61, 0.54, 0.47, 0.36 and 0.15 kGy for *Salmonella* spp., *S. aureus*, *L. monocytogenes*, *E. coli* O157:H7, and *Y. enterocolitica*, respectively when inoculated onto the three product types. These results indicate that irradiation may be an effective means for inactivating common foodborne pathogens including *Salmonella* spp, *S. aureus*, *L. monocytogenes*, *E. coli* O157:H7 and *Y. enterocolitica* in complex RTE food products such as 'heat and eat" sandwich products.
Published by Elsevier Ltd.

Keywords: Gamma; Ionizing; Irradiation; Sandwiches; Complex; Ready-to-eat

1. Introduction

Tracking of consumer food purchases in the USA indicates an increasing trend in the consumption of ready-to-eat (RTE) "convenience" types of foods (Anonymous, 2001, 2003). One of the most popular types of convenience foods is the RTE sandwich product. These types of products can vary in type from "heat and eat" products such as "hot dogs" and "burgers" to more exotic RTE sandwiches such as the popular "wrap" type of product or "submarine" and "deli" sandwiches. Sales of these sandwich products account for 32% of sales from vending machines, and are a multi-billion dollar annual business in the USA (Anonymous, 2001). In addition, consumers visiting retail food outlets including convenience stores and supermarkets will find a wide variety of complex RTE food products available.

☆Mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the US Department of Agriculture.

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0969-806X/$ - see front matter Published by Elsevier Ltd.
doi:10.1016/j.radphyschem.2005.12.036

Examination of refrigerated RTE sandwich products available for purchase from either retail stores or vending machines indicates they have relatively short shelf-lives that range from as little as 24 to 96 h. Because RTE sandwiches are multi-ingredient products that require assembly by humans, they are especially susceptible to post-process contamination by pathogenic bacteria (Wilson, 1996). Such products, which require little of no preheating before consumption, are therefore a potential reservoir for foodborne pathogens and a source of foodborne illness.

Examination of food product recalls and foodborne illness outbreaks listed on Foods and Drug Administration (FDA), USDA Food Safety Inspection Service (FSIS), and Centers for Disease Control and Prevention (CDCP) websites indicates a wide variety of pathogenic bacteria and viruses are associated with those events (Anonymous, 2002; USDA FSIS, 2004; US FDA, 2004). Similar trends have been observed in European nations (Euroserveillance Weekly, 2005). Consumers, especially those who are immuno-compromised, which comprise approximately 20% of the US population (Gerba et al., 1996; Smith, 1998), would benefit from the commercial availability of radiation-pasteurized complex RTE food products. Because of the potential health benefit of irradiating complex RTE foods, a petition was filed via the National Food Processors Association with the US FDA to allow their irradiation (NFPA, 1999). No approval or disapproval of the petition, or parts of the petition, has been issued in the 7 years since its filing.

Ionizing radiation is an effective intervention that can be used to reduce pathogen levels on raw meat and poultry (Thayer et al., 1995), processed meats (Sommers et al., 2004), cheeses (Bougle and Stahl, 1994; Ennhar et al., 1994; Cecchi et al., 1996), minimally processed fruit vegetable products (Niemira et al., 2003b; Prakash and Foley, 2004). Recently, the CDC estimated that if 50% of the poultry, ground beef, pork, and processed meats in the US were irradiated there would be a 25% reduction in the morbidity and mortality associated with those products (Tauxe, 2001). However, the estimates provided by the CDC do not account for the benefits of using irradiation to treat other foods, such as fresh products and complex RTE products that are popular with consumers.

There have been a limited number of studies investigating the use of ionizing radiation to reduce pathogen levels on complex RTE products such as sandwiches and ready meals (McAteer et al., 1995; Foley et al., 2001; IAEA, 2003). The purpose of this study was to investigate the feasibility using ionizing radiation to inactivate pathogenic bacteria including *Listeria monocytogenes*, *Staphylococcus aureus*, *Salmonella* spp., *Escherichia coli* O157:H7, and *Yersinia enterocolitica* on heat and eat sandwich products including a precooked frankfurter on a bun, a precooked cheeseburger on a bun, and a precooked vegetarian burger on a bun.

2. Materials and methods

2.1. Bacterial strains

L. monocytogenes strains H7762, H7962, H7969 were obtained from the CDCP (Atlanta, GA). *S. aureus* strains 25923, 13565, and 14458; *S.* Enteriditis 13076, *S.* Typhimurium and *S.* Newport 6962, *Y. enterocolitica* 51871; and *E. coli* O157:H7 35150 were obtained from the American Type Culture Collection (Manassa, VA). *Y. enterocolitica* strains GER and PT18 were obtained from Dr. Saumya Bhaduri (USDA, Wyndmoor, PA). *E. coli* O157:H7 strain ENT C9490 was obtained from the CDCP (Atlanta, GA), and strain 93-437 from the Oregon Public Health Laboratory (Portland, OR). Identity of the isolates were confirmed by Gram Stain, followed by analysis with Gram positive or negative identification cards using the Vitek Automicrobic System (bioMerieux Vitek Inc., Hazelwood, MO). The bacterial strains were cultured on tryptic soy agar (TSA) (BBL/Difco Inc., Sparks, MD) at 37 °C and maintained at 0–4 °C, until use.

2.2. RTE foods

RTE foods were purchased from local retail outlets. Bread products included fortified white bread hamburger and frankfurter rolls, precooked beef, and vegetarian patties, which were frozen, were allowed to thaw prior to assembly of the actual "burger" product. The assembled burgers consisted of a bun with either a beef or vegetarian patty, and a slice of processed-pasteurized "American-style" cheese. Frankfurters were thawed and then cooked to an internal temperature of 70 °C before being placed on a frankfurter roll. Examination of frankfurters sold in vending machines indicated the use of reheated (cooked) frankfurters in the actual commercial product.

2.3. Preparation of inoculum

The procedure of Sommers and Thayer (2000) for inoculation, irradiation, and enumeration of bacteria was utilized. Each bacterial strain was cultured independently in 100-mL tryptic soy broth (BBL/Difco Laboratories, Sparks, MD) in baffled 500-mL Erlenmeyer culture flasks at 37 °C (150 rpm) for 18 h. The cells (30 mL culture per isolate) were then pelleted by centrifugation and then resuspended as a "same species mixture" in a total 9-mL of Butterfield's phosphate buffer (BPB) (Applied Research Institute, Newtown, CT).

2.4. Inoculation

The assembled products were cut into equal quarters and then inoculated with 0.1 mL (10^8 CFU) of bacterial species mixture, pipetted onto the multiple components of the RTE products and then distributing onto a larger surface area using a sterile cotton swab moistened with BPB. Non-inoculated products were also assembled to measure background microflora. The inoculated quarters were then placed in polyethylene No. 400 Stomacher bags (Seward Ltd., London, UK) and the bags sealed with an A300 Packager (Kansas City, MO) under aerobic conditions. The inoculated products, and non-inoculated products used to measure background microflora, were refrigerated (0–4 °C, approximately 60 min).

2.5. Gamma irradiation

A Lockheed Georgia Company (Marietta, GA) self-contained ^{137}Cs radiation source was used for all exposures. The radiation source consisted of 23 individually sealed source pencils placed in an annular array. The 22.9 cm × 63.5 cm cylindrical sample chamber was located central to the array when placed in the operating position. The dose rate was 0.092 kGy/min. The temperature during irradiation was maintained at 4.0 (±1.0)°C by the gas phase of a liquid nitrogen source that was introduced directly into the top of the sample chamber. To ensure uniform radiation dose was delivered, sample bags were placed centrally and vertically within the cylindrical chamber, and as a result the dose-uniformity ratios (DURs) were less than 1.1: 1.0 for each of the three sample types used in the study. The temperature was monitored using two thermocouples placed on the side of the sample bags. The dose delivered was verified by use of 5 mm alanine pellet dosimeters that were attached to the side of the sample bags, which were then measured using a Bruker EMS 104 EPR Analyzer (Billerica, MA). Recorded doses were typically ±5% of the target doses. Radiation doses utilized ranged from 0 to 2.5 kGy depending on the bacterium.

2.6. Enumeration of bacteria

Following irradiation, the samples were assayed for surviving bacteria by standard pour plate procedures. About 100 mL of sterile BPB was added to a No. 400 Stomacher bag that contained a 5-g inoculated sample and the sample mixed by stomaching for 90 s. The samples were then serially diluted in BPB, using 10-fold dilutions, and 1 mL of diluted sample was pour plated using Palcam agar (*Listeria*), Baird-Parker agar (*S. aureus*) Hekteon agar (*Salmonella* spp.), or TSA (BBL/Difco Laboratories, Sparks, MD) for *E. coli* O157:H7 and *Y. enterocolitica*. Three 1 mL aliquots were plated per dilution. The plates were then incubated for 24–48 h at 37 °C prior to enumeration. Non-inoculated non-irradiated samples were used to determine aerobic plate counts by pour plating on TSA, and indicated that background microflora levels were less than $2 \log_{10}$ CFU/g, which did not interfere with determination of D-10 values for *E. coli* O157:H7 or *Y. enterocolitica*.

2.7. D-10 values

The average CFU/g of an irradiated sample (N) was divided by the average CFU/g of the untreated control (N_o) to produce a survivor ratio (N/N_o). D-10 value is defined as the radiation dose required to achieve a 90% reduction in viable microorganism. D-10 values were determined by calculating the reciprocal of the slope of the $\log_{10}$ (N/N_o) ratios versus irradiation dose (Diehl, 1995).

2.8. Statistical analysis

Each experiment was conducted independently three times. Determination of D-10 values, descriptive statistics, analysis of variance (ANOVA), and analysis of covariance (ANCOVA) were completed using Microsoft Excel Office 2000 (Microsoft Corp., Redmond, WA).

3. Results and discussion

RTE foods are susceptible to post-process contamination by foodborne pathogens, and been associated with occasional foodborne illness outbreaks and product recalls (Anonymous, 2002; USDA FSIS, 2004; US FDA, 2004). Development of radiation-pasteurized RTE foods specifically for the immunocompromised portion of the population is therefore a realistic and attainable goal for the radiation processing industry.

Ionizing radiation is able to inactivate foodborne pathogens on a variety of food products and can serve as a final critical control point to ensure the microbiological safety of RTE foods. Thayer et al. (1995) determined that ionizing radiation could inactivate pathogens from a variety of raw meat products. Cecchi et al. (1996) found that ionizing radiation could significantly reduce levels of multiple pathogen species on cheese products. The radiation doses needed to reduce levels of the psychrotroph *L. monocytogenes* from cheese products is dependent on the cheese type (Bougle and Stahl, 1994; Ennhar et al., 1994; Tsiotsias et al., 2002). Ionizing radiation can also be used to reduce bacterial load on breads with little effect on product quality (Grecz et al. 1985). While the database on radiation inactivation of foodborne pathogens,

Table 1
Radiation resistance of foodborne pathogens inoculated onto multi-component ready-to-eat food products

	E. coli O157H7	*Salmonella* spp.	*Y. enterocolitica*	*L. monocytogenes*	*S. aureus*
Hot dog	0.39^{a} (±0.03)	0.61^{a} (±0.03)	0.23^{b} (±0.01)	0.43^{a} (±0.01)	0.48^{a} (±0.01)
Beef cheese burger	0.37^{a} (±0.02)	0.63^{a} (±0.02)	0.11^{a} (±0.02)	0.53^{b} (±0.02)	0.57^{a} (±0.02)
Vegetarian cheese burger	0.32^{a} (±0.03)	0.59^{a} (±0.02)	0.10^{a} (±0.02)	0.46^{a} (±0.04)	0.57^{a} (±0.03)
Mean	0.36 (±0.02)	0.61 (±0.01)	0.15 (±0.02)	0.47 (±0.02)	0.54 (±0.02)

D-10 values have followed the standard error of the mean (SEM). D-10 values that are statistically the same within each column, as determined by Duncan's range of the means test, are designated by the same letter value. Each experiment was conducted independently three times ($n = 3$). Mean values were determined using the pooled D-10 values for each microorganism ($n = 9$).

especially *L. monocytogenes*, on single-component RTE foods is extensive, the information on the radiation doses need to inactivate on multiple pathogens on complex RTE foods is more limited (McAteer et al., 1995; Foley et al. 2001; Clardy et al., 2002; Lamb et al., 2002; IAEA, 2003).

In this study, the average radiation resistances of foodborne pathogens inoculated onto complex RTE foods were *Salmonella* spp (0.61 kGy) > *S. aureus* (0.54 kGy) > *L. monocytogenes*(0.47 kGy) > *E. coli* O157:H7 (0.36 kGy) > *Y. enterocolitica* (0.15 kGy), with the differences in radiation resistance for each pathogen being statistically different ($n = 9$, $\alpha = 0.05$) (Table 1) than the other pathogen species. There were also statistically significant differences in the radiation resistances of pathogens depending on the menstrum (Table 1). While the D-10 values for *E. coli* O157:H7, *S. aureus*, and *Salmonella* spp. were equivalent when inoculated onto the three products, the D-10 value for *Y. enterocolitica* inoculated onto the frankfurter product was greater than that on the beef cheeseburger or vegetarian cheeseburger ($n = 3$, $\alpha = 0.01$). The D-10 value for *L. monocytogenes* inoculated onto the beef cheeseburger was greater than that inoculated onto the frankfurter product and vegetarian cheeseburger ($p < 0.07$ when determined by ANOVA, $p < 0.05$ when determined by ANCOVA).

Thayer et al. (1995) examined the radiation dose required to inactivate multiple foodborne pathogens in a variety of refrigerated raw meat products, finding the order of radiation resistance to be *Salmonella* spp. > *S. aureus* > *L. monocytogenes* > *E. coli* O157:H7. Sommers and Novak (2002) found the radiation resistance of *Y. enterocolitica* to be less than that reported for other common pathogens suspended in raw ground pork. While the radiation resistance of foodborne pathogens inoculated onto leafy vegetables is typically less than that when inoculated onto meat or processed meat, the radiation sensitivities of the pathogens are similar in order, or *Salmonella* spp. > *L. monocytogenes* > *E. coli* O157:H7 (Niemira et al., 2002; Niemira, 2003a, b). The results obtained in this study confirm the "order of radiation resistances" for these common foodborne pathogens within food product groups, with *Salmonella* spp. being the most radiation resistant.

While it is recognized that irradiation is an effective intervention technology for the purpose of pathogen reduction, the radiation dose utilized for pathogen reduction cannot, by definition, significantly impact product quality factors such as taste and aroma if the process is to be used commercially. Sommers and Boyd (2005) found that irradiation (2.5 kGy) of turkey and cheese tortilla wraps, which included a processed-pasteurized American cheese product, as opposed to a traditional Swiss or Provolone cheese, did not change the appearance or aroma of the product. Clardy et al. (2002) and Lamb et al. (2002) found that irradiation (<4 kGy) of frozen sandwiches that included a RTE meat and cheese product produced an organoleptically acceptable product. Chen et al. (2004) found that irradiation of frankfurters (3.5 kGy) did not adversely affect their sensory quality. Grecz et al. (1985) found that irradiation (6 kGy) did not affect the quality of irradiated Arabic breads. Zhu et al. (2005) found that irradiation of ham (2 kGy) had minimal impact on the quality of ham that contained the antimicrobials, sodium lactate and sodium diacetate. Thus, the limited research that has been conducted indicates that irradiation of RTE sandwiches, including heat and eat types or products, to inactivate foodborne pathogens while maintaining acceptable product quality is a feasible endeavor.

4. Conclusion

Ionizing radiation, at doses below 10 kGy commonly associated with pasteurization as opposed to sterilization, can significantly reduce levels of *Salmonella*, *S. aureus*, *L. monocytogenes*, *E. coli* O157:H7, and *Y. enterocolitica* in ready-to-eat (RTE) complex food products such as heat and eat sandwiches. Ionizing radiation, used as a terminal intervention as part of a Hazard Analysis and Critical Control Point Plan

(HACCP), should be considered as a means of providing microbiologically safer complex RTE food products to "at risk" immuno-compromised populations in order to reduce the incidence of foodborne infections, hospitalizations, and mortality.

Acknowledgment

We thank K. Snipes for completion of the technical work for this study.

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